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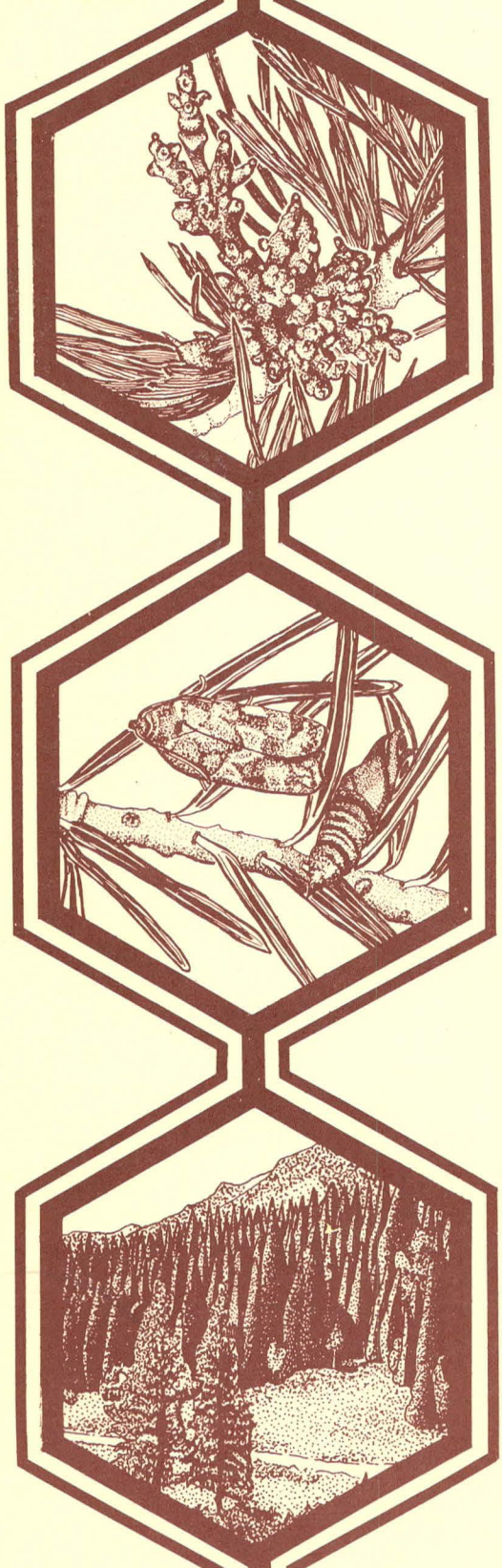

Comparison of Stain Measurements on Spray Deposit Cards by the Quantimet 720 Image Analyzer

Methods Application Group
Forest Insect and Disease Management
Forest Service, USDA
Davis, California

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Report No. 77-1
July 1977

COMPARISON OF STAIN MEASUREMENTS ON SPRAY DEPOSIT CARDS BY THE QUANTIMET 720 IMAGE ANALYZER [1/]

Lynne Whyte, John W. Barry, and Robert Young [2/]

ABSTRACT

An evaluation was conducted to compare percent variation in measurement of stain diameters on Kromekote cards by four different Quantimet 720 Image Analyzers. Percent variability by stain size classes was determined for each unit and observer. Results show that variation was greatest when measuring stain diameters between 40 and 200 micrometers. Generally, as stain diameters increased in size, percent variation among Quantimet units decreased.

INTRODUCTION

Spray deposit assessment plays an integral role in the evaluation of field experiments, pilot projects and operational projects of insecticides against forest insects. It is instrumental in the determination of quality and quantity of spray application.

The USDA Forest Service (USFS) has been utilizing the Quantimet 720 Image Analyzer to assess spray deposit cards since 1974. This instrument rapidly counts and sizes stains on Kromekote cards automatically, whereas non-automated or visual evaluation of the cards is a tedious, time consuming, expensive process that can introduce a variety of human errors.

A comparative evaluation was conducted in response to suggestions made by participants of the 1976 Spray Deposit Assessement Workshop (Anonymous 1976) held at Davis, California, during March 1976. This workshop was sponsored by the USFS, Forest Insect and Disease Management, Methods Application Group (MAG), USDA Douglas-fir Tussock Moth Accelerated Research and Development Program, and the USDA Gypsy Moth Accelerated Research and Development Program. During this workshop, it was suggested that an evaluation be conducted comparing measurements of stains on white Kromekote cards from four different Quantimet 720 Image Analyzers.

1/ Mention of a commercial product is for reference only and does not imply endorsement by USDA.

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Objectives of this evaluation were to:

(1) Measure magnitude and direction of bias (if any) for both Quantimet and visual observations.
(2) Measure and determine percent variation in size class measurements between Quantimet and visual measurements.

Description of Quantimet 720 Image Analyzers

Image Analyzers have been used to measure and record image information from optical and electron microscopes, photographs, negatives, cine and strip films, slides, radiographs, and various types of spray deposit substrates (i.e. cards, glass slides). Objects and features of interest within the field can be selected automatically, or by using a light-pen, counted and classified according to shape, size density, orientation, and morphological features.

When using the Quantimet to count and size stains on Kromekote cards, the card is mounted on a macroviewer or microscope consisting of a lens system of suitable magnification. The image is scanned using a high resolution Plumbicon or Vidicon scanner specifically developed for image analysis.

The image is displayed on a cathode ray tube which is an integral part of the system. When stains are selected for measurement, a superimposed display presents the field of view to the operator along with a digital presentation of the measurements. Furthermore, special marker "flags" indicate on the display those stains which are being counted or selected by criteria such as size, shape or density.

Stains are selected for measurement by choosing the appropriate gray level threshold. That is, they can be selected because their contrast is sufficiently different from that of the background or card material.

The digital signal obtained by the detection procedure described above is then fed into several computing modules which provide the data output. Numerical data may be viewed on the cathode ray tube display and fed to printers, calculators and computers.

In the assessment of droplet stains, experience has shown that for maximum accuracy and reproducibility over a wide range of stain densitites, use of the configuration described in the Appendix is recommended.

METHODS

Preparation of Test Cards

A total of 49 droplets representing three separate formulations were generated in the USFS, Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory in Corvallis, Oregon, and deposited on white Kromekote cards. Cards A through L were stained with a formulation of

water and 0.5% concentration of Rhodamine B Extra S dye. Card M was stained with droplets of tripropylene-monomethyl glycol ether (TPM) and 0.5% Rhodamine B Extra Base dye. Cards N and O were stained with droplets consisting of 1 part TPM and 9 parts fuel oil and 0.1% Rhodamine B Extra Base dye.

The forty-nine stains, ranging in size from approximately 40 to 3200 micrometers in diameter were produced on white Kromekote cards by a vibrating reed apparatus (Davis 1951, Rayner & Hurtig 1954, and Wolf 1961).

Each stain was identified and its diameter sized in micrometers by five operators using Quantimets designated as Quantimets A, B, C_1, C_2 and D. Four individuals experienced in measuring stains with non-automated optical instruments also measured the stains.

Quantimet A used a Plumbicon scanner and a 63 mm focal length lens, one picture point = 0.038 mm. Quantimet B used a Plumbicon scanner and a 63 mm focal length lens, one picture point = 0.045 mm. Quantimet C, operators 1 and 2 (C_1 and C_2 respectively) used a Vidicon scanner and a 32 mm focal length lens, 1 picture point = 0.091 mm. Quantimet D used a microscope with a Vidicon scanner and an 8 mm focal length lens, 1 picture point = 0.0056 mm. A picture point is the minimum resolution and it is dependent upon the lens system.

Three of the visual sizers used a Bausch and Lomb 7X measuring magnifier, with a 0.1 mm division on the scale. The fourth observer used a Zeiss dissecting microscope at 30X magnification.

Data Analysis

Procedure used to analyze the data is similar to a quality control technique where each measured stain size is compared to a standard size. A standard or "true stain size" was derived since a standard stain size was not known ahead of time. The derived standard stain size was determined by eliminating the highest and lowest stain measurement and averaging the remaining measurements. The derived standard stain size was determined for each stain (Table 1) using both the Quantimet and visual measurements.

The variables in the analysis were:

(1) Measured stain diameter
(2) Derived standard stain size
(3) Difference: Measured stain size - derived standard stain size
(4) Percent difference:

$$\frac{\text{Measured stain size - derived standard stain size}}{\text{derived standard stain size}} \times 100$$

A mean and standard error for each of the four variables were computed for all 49 stains and by the following five stain size categories:

Size Category	Stain Diameter (μm)
1	40 - 200 μm
2	201 - 500 μm
3	501 - 700 μm
4	701 - 1000 μm
5	1001 - 3200 μm

There were five instances where observers were not able to size particular stains because they were undetectable. Missing values were eliminated from the analyses.

The magnitude and direction of bias were determined for each stain size, each size category, and for all measurements. Bias is defined as the distance of the estimated mean from the true mean. Estimates of means that are unbiased in a sampling framework might appear to be "good" in the sense of having a relatively low sampling error (precision), but could be much different from the true mean (accuracy). Errors in measurements will produce biases. The serious problem occurs when these measurement errors are either consistently higher or lower than the true mean.

The test statistic "t" was used to determine if the bias was negligible or not. The hypothesis tested is that the bias for each observer is zero.

H_o: Measured - derived standard = 0, for each size category and combined

$$\bar{d} = \sum_{i=1}^{n} \frac{(\text{measured - derived standard})}{n}$$

$$t = \bar{d} \; / \; S.E._{\bar{d}}$$

$$S.E._{\bar{d}} = \sqrt{\frac{\Sigma d_i^2 - (\Sigma d_i)^2/n}{n(n-1)}}$$

The α error term for the test was .05.

RESULTS AND DISCUSSION

Percent variation between Quantimet measurements of stain sizes was greatest when measuring stains between 40 and 200 μm. Generally, as the stains increased in diameter, percent variation among measurements decreased with the exception of size category 4 (701 - 1000 μm) which had a 2.8% increase, (Figure 1, Tables 1 and 2), by one Quantimet. Differences between measured stain diameter and derived standard stain values are presented in Table 3.

TABLE 1. - RESULTS OF QUANTIMET AND VISUAL ANALYSES OF STAIN DIAMETERS.

Drop I.D.	Quantimet A	Quantimet B	Quantimet C_1 Operator 1	Quantimet C_2 Operator 2	Quantimet D	Hand Sizer A	Hand Sizer B	Hand Sizer C	Hand Sizer D	Derived Standard Stain Size Value
A1	3154	2960	2937	2938	3050	3000	2900	2950	2000	2976
B1	2508	2340	2298	2313	2413	2400	2400	2400	2410	2382
C1	1900	1740	1701	1722	1827	1700	1800	1800	1780	1761
D1	570	500	493	499	559	550	550	600	560	541
D2	836	770	760	762	788	800	800	850	800	794
D3	988	920	876	879	905	900	900	1000	940	919
D4	456	360	356	365	413	400	450	485	410	408
D5	456	360	376	373	436	400	400	450	420	408
E1	684	590	585	581	653	600	700	650	660	632
E2	646	610	595	588	642	600	600	625	640	616
E3	646	590	593	585	637	650	650	650	640	630
E4	646	590	599	595	704	650	600	640	660	627
E5	684	590	597	590	665	650	600	650	680	633
E6	684	590	617	593	648	650	600	650	660	631
E7	684	590	599	594	665	600	600	675	680	630
F1	798	720	680	697	765	750	700	750	750	733
F2	836	740	707	710	760	750	750	800	780	756
G1	532	450	454	450	503	500	500	500	500	487
G2	456	440	368	372	436	400	400	430	440	417
G3	494	430	432	421	480	450	450	500	480	460
H1	456	340	358	350	397	400	400	420	410	391
H2	456	360	351	361	402	400	400	420	440	398
H3	456	320	362	364	408	400	400	450	400	403
I1	456	320	364	336	402	400	400	400	420	389
I2	418	360	321	299	385	350	350	400	410	368
J1	342	230	234	234	285	250	250	300	310	266
J2	342	200	240	234	302	300	250	300	310	277
J3	342	200	236	223	263	250	250	300	310	262
J4	304	180	248	236	285	259	259	300	310	268
J5	266	140	212	188	223	200	200	250	280	220
K1	266	90	121	125	145	100	150	180	190	144
K2	342	90	121	129	151	125	150	180	190	149
K3	304	90	125	129	162	125	150	190	190	153
K4	304	90	125	121	123	125	150	190	190	146
K5	152	90	121	121	145	150	150	180	190	146

TABLE 1. - RESULTS OF QUANTIMET AND VISUAL ANALYSES OF STAIN DIAMETERS (CONTINUED).

Drop I.D.	Quantimet A	Quantimet B	Quantimet C_1 Operator 1	Quantimet C_2 Operator 2	Quantimet D	Hand Sizer A	Hand Sizer B	Hand Sizer C	Hand Sizer D	Derived Standard Stain Size Value
K6	152	90	104	125	156	125	150	180	190	142
K7	152	90	104	108	134	100	150	190	190	134
L1	190	70	99	108	112	100	100	130	160	116
L2	190	40	94	82	112	100	100	150	160	114
L3	190	40	117	82	not sized	not sized	not sized	not sized	not sized	100
L4	190	40	136	162	123	100	100	130	160	130
L5	228	70	143	188	128	100	100	190	160	144
L6	266	70	143	136	117	100	100	150	160	129
M1	2432	2300	1857	2271	2424	2400	2400	2450	2380	2375
N1	722	660	not sized	555	880	700	800	775	750	717
N2	570	660	not sized	581	750	700	800	800	750	707
O1	1330	1360	not sized	1227	1500	1400	1500	1600	1530	1437
O2	1406	1380	not sized	1257	1410	1500	1550	1600	1520	1461
O3	1482	1380	not sized	1234	1320	1550	1500	1600	1520	1459

The Standard Stain Size Values were obtained by eliminating the high and low values of stain estimates and computing a mean for the remaining 7 observations.

TABLE 2. - MEASURED STAIN DIAMETERS (μm) BY SIZE CATEGORIES.

Size Category	Statistic	QUANTIMET					VISUAL				Derived Standard Stain Size
		A	B	C_1	C_2	D	A	B	C	D	
40 - 200 μm	n	13	13	13	13	12	12	12	12	12	13
	$\bar{x}$	225.0	73.9	119.5	124.3	134.0	112.5	129.2	170.0	177.5	134.4
	S.E.	17.98	5.83	4.39	7.92	5.02	4.87	7.43	6.74	4.46	4.43
201-500 μm	n	15	15	15	15	15	15	15	15	15	15
	$\bar{x}$	415.5	312.7	327.5	320.4	374.7	356.7	356.7	393.7	392.7	361.1
	S.E.	19.79	25.66	19.54	20.53	21.45	22.29	23.84	21.35	18.03	21.12
501-700 μm	n	8	8	8	8	8	8	8	8	8	8
	$\bar{x}$	655.5	581.3	584.8	578.1	646.6	618.8	612.5	642.5	647.5	617.5
	S.E.	13.91	11.87	13.49	11.43	14.52	13.15	15.67	7.79	13.59	11.09
701-1000 μm	n	6	6	4	6	6	6	6	6	6	6
	$\bar{x}$	791.7	745.0	755.8	697.3	808.0	766.7	791.7	829.2	795.0	771.0
	S.E.	56.72	39.30	43.39	48.70	27.39	30.73	27.13	36.75	30.19	32.21

TABLE 2. - MEASURED STAIN DIAMETERS (μm) BY SIZE CATEGORIES (CONTINUED).

Size Category	Statistic	QUANTIMETS A	B	C_1	C_2	D	VISUAL A	B	C	D	Derived Standard Stain Size
1001-3200 μm	n	7	7	4	7	7	7	7	7	7	7
	$\bar{x}$	2030.3	1922.9	2198.3	1851.7	1992.0	1992.8	2007.1	2057.1	2020.0	1979.6
	S.E.	260.49	235.61	276.80	254.06	246.06	230.02	211.13	204.83	220.38	228.38
Total	n	49	49	44	49	48	48	48	48	48	49
	$\bar{x}$	680.9	576.1	521.8	575.4	649.9	629.2	637.5	676.3	668.9	624.2
	S.E.	91.46	91.56	89.63	87.43	93.60	93.72	93.20	93.40	91.94	90.99

TABLE 3. - DIFFERENCES, MEASURED STAIN DIAMETER TO DERIVED STANDARD STAIN VALUE.

Size Category	Statistic	QUANTIMET A	QUANTIMET B	QUANTIMET C_1	QUANTIMET C_2	QUANTIMET D	VISUAL A	VISUAL B	VISUAL C	VISUAL D
40 - 200 µm	n	13	13	13	13	12	12	12	12	12
	$\bar{x}$	90.7	-60.5	-14.9	-10.1	-3.3	-24.8	-8.1	32.8	40.3
	S.E.	16.45	3.47	4.95	6.53	2.96	3.86	5.32	4.33	3.02
	t	5.51	-17.43	-3.01	-1.54*	-1.10*	-6.41	-1.52*	7.56	13.32
201-500 µm	n	15	15	15	15	15	15	15	15	15
	$\bar{x}$	54.4	-48.4	-33.6	-40.7	13.6	-4.4	-4.4	32.6	31.6
	S.E.	3.51	7.63	3.24	2.59	2.30	3.39	4.53	4.28	4.21
	t	15.50	-6.34	-10.37	-15.71	5.91	-1.30*	-.97*	7.62	7.51
501-700 µm	n	8	8	8	8	8	8	8	8	8
	$\bar{x}$	38.0	-36.3	-32.8	-39.4	29.1	1.3	-5.0	25.0	30.0
	S.E.	5.73	4.37	4.18	2.62	7.53	8.27	12.56	6.17	4.74
	t	6.63	-8.30	-7.83	-15.03	3.87	.15*	-.39*	4.05	6.33
701-1000 µm	n	6	6	4	6	6	6	6	6	6
	$\bar{x}$	20.7	-26.0	-44.8	-73.7	-37.0	-4.3	20.7	58.2	24.0
	S.E.	33.36	8.95	4.13	22.80	26.75	5.62	21.98	11.01	5.24
	t	.62*	-2.91	-10.85	-3.23	1.38*	-.77*	.94*	5.28	4.58

TABLE 3. - DIFFERENCES, MEASURED STAIN DIAMETER TO DERIVED STANDARD STAIN VALUE (CONTINUED).

Size Category	Statistic	QUANTIMET A	B	C_1	C_2	D	VISUAL A	B	C	D
1001 - 3200 µm	n	7	7	4	7	7	7	7	7	7
	$\bar{x}$	50.7	-56.4	-176.8	-127.9	12.4	13.3	27.6	77.6	40.4
	S.E.	39.49	10.45	114.13	31.23	29.78	19.48	19.56	27.33	11.92
	t	1.28*	-5.43	-1.55*	-4.09	.42*	.68*	1.41*	2.84	3.39
Total	n	49	49	44	49	48	48	48	48	48
	$\bar{x}$	56.7	-48.1	-42.0	-48.8	14.7	-5.9	2.37	41.1	33.8
	S.E.	8.54	3.50	11.44	7.54	5.68	3.79	4.99	5.09	2.54
	t	6.64	-13.74	-3.67	-6.47	2.59	-1.57*	.47*	8.07	13.32

* An asterisk has been placed on those values not significantly different from zero (95%) level.

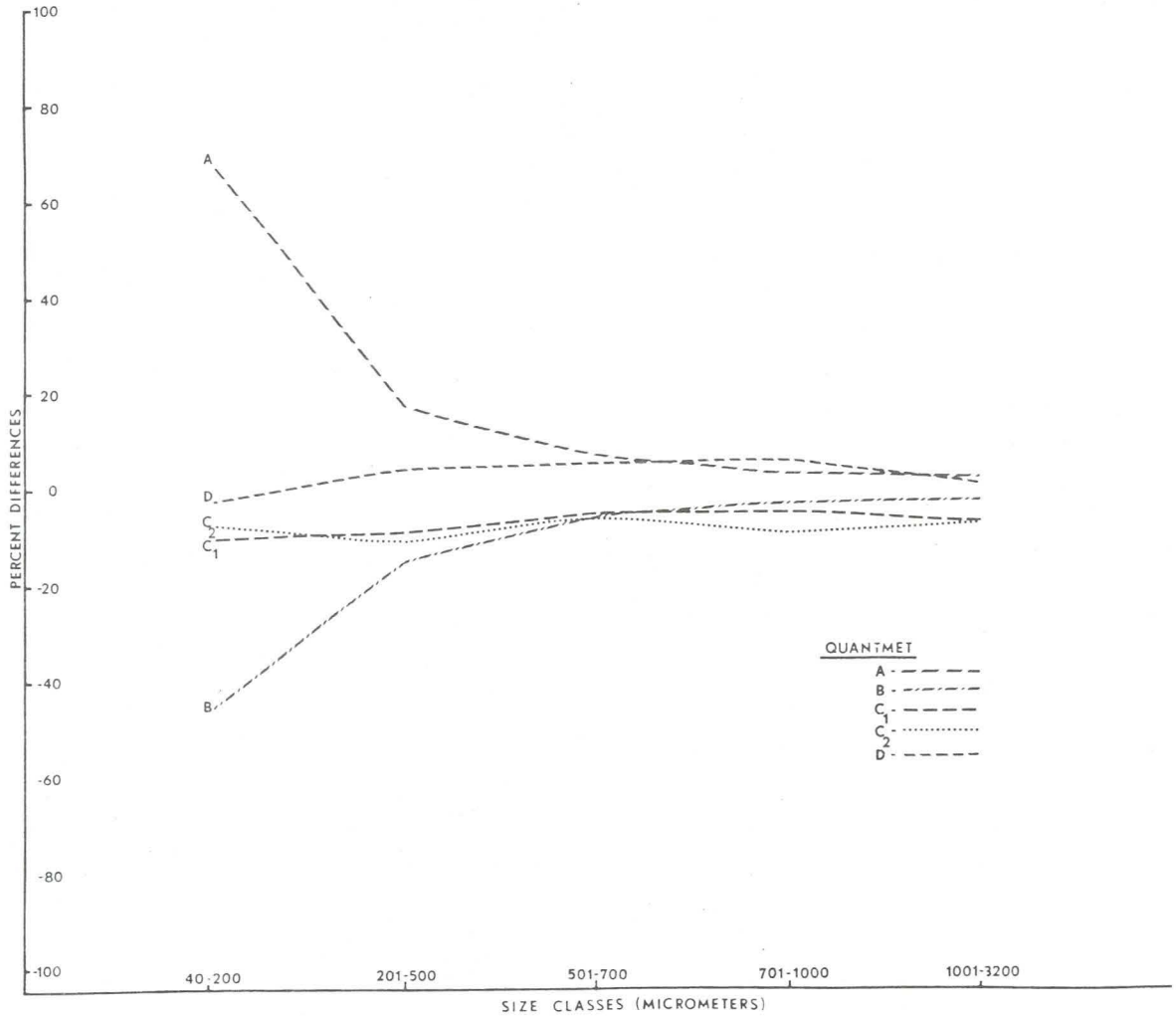

FIGURE 1. - PERCENT DIFFERENCES FROM THE DERIVED STANDARD SIZE - QUANTIMET ANALYSES.

The magnitude of spread in percent difference was 113.3% in size category 1 (40 - 200 µm), 31.5% in size category 2 (201 - 500 µm), 12.4% in size category 3 (501 - 700 µm), 15.2% in size category 4 (701 - 1000 µm) and 9.5% in size category 5 (1001 - 3200 µm), (Table 4).

Quantimets C_1, C_2 and D were not significantly different from each other using percent variation as the variable. These Quantimets consistently sized below the derived standard. Quantimets A and B differed from each other and from C_1, C_2, and D. Quantimet A consistently sized above the derived standard, having the highest overall percent difference. Quantimet D was generally the closest to the derived standard. Quantimet D had the lowest percent difference, 2.06% for all 49 observations ranging from a high of 5.2% difference for size Category 4 (701 - 1000 µm) to a low of .09% for sizes between 1001 - 3200 µm.

Percent variation between visually determined measurements from the estimated derived standard stain size was also greatest with stains between 40 and 200 µm. The percent variation among measurements decreased as the stain sizes increased (Figure 2).

CONCLUSIONS AND RECOMMENDATIONS

Variation in results of measuring stain diameters on Kromekote cards can be expected among different Quantimets having different sensitivities, and auxilliary equipment. Different levels of experience among operators also may contribute to variation. Variation is likely to be greater for stains less than 500 µm.

We recommended that a standard calibration procedure using reference samples be developed for use by each Quantimet unit assessing stains on Kromekote cards.

ACKNOWLEDGEMENTS

Special thanks are given to those individuals and organizations providing their support and analyses as follows: Brian C. Partridge, Cambridge Instrument Company, Mountain View, California; Dr. Clarence H. Thompson, USFS Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Corvallis, Oregon; Kaye A. Johnson, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico; and Robert T. Stormont, University of California, Davis Campus, Davis, California, for providing Quantimet 720 Image Analyses; Richard A. Waite, USFS Forestry Sciences Laboratory, Corvallis, Oregon, who prepared the test Kromekote cards and hand sized the stains using a binocular compound microscope, and Michael J. Haskett, USFS Pacific Northwest Forest and Range Experiment Station, Field Evaluation of Chemical Insecticides (Project 2206) Davis, California, who hand sized the stains using a measuring magnifier.

TABLE 4. - PERCENT DIFFERENCES IN QUANTIMET AND VISUAL STAIN MEASUREMENTS FROM DERIVED STANDARD STAIN SIZE VALUE.

Size Category	Statistic	QUANTIMETS					VISUAL			
		A	B	C_1	C_2	D	A	B	C	D
40 - 200 µm	n	13	13	13	13	12	12	12	12	12
	$\bar{x}$	67.5	-45.8	-10.3	-7.7	-2.5	-18.0	-6.4	23.7	29.7
	S.E.	11.30	3.28	3.78	4.85	2.07	2.68	3.93	3.10	2.47
	t	5.97	-13.97	-2.73	-1.59*	-1.19*	-6.72	-1.62*	7.63	12.01
201-500 µm	n	15	15	15	15	15	15	15	15	15
	$\bar{x}$	16.3	-15.2	-9.3	-11.7	3.9	-1.6	-2.0	-9.6	10.2
	S.E.	1.78	2.90	.81	.82	.75	1.13	1.34	1.25	1.9
	t	9.16	-5.24	-11.49	-14.28	5.21	-1.38*	-1.46*	7.66	5.31
501-700 µm	n	8	8	8	8	8	8	8	8	8
	$\bar{x}$	6.1	-5.9	-5.4	-6.4	4.7	.2	-.8	4.2	4.8
	S.E.	.89	.72	.75	.45	1.19	1.32	1.99	1.14	.73
	t	6.89	8.18	-7.15	-14.20	3.94	.17*	-.39*	3.65	6.61
701-1000 µm	n	6	6	4	6	6	6	6	6	6
	$\bar{x}$	2.3	-3.6	-5.7	-10.0	5.2	-5.2	3.0	7.5	3.2
	S.E.	4.55	1.26	.71	3.31	3.70	.73	3.04	1.46	.77
	t	.51	-2.86	-8.06	-3.02	1.41*	.72*	.99*	5.16	4.15

TABLE 4. - PERCENT DIFFERENCES IN QUANTIMET AND VISUAL STAIN MEASUREMENTS FROM DERIVED STANDARD STAIN SIZE VALUE (CONTINUED).

Size Category	Statistic	QUANTIMETS					VISUAL			
		A	B	C_1	C_2	D	A	B	C	D
1001 - 3200 µm	n	7	7	4	7	7	7	7	7	7
	$\bar{x}$	1.7	-3.3	-7.6	-7.9	.1	.7	2.1	5.1	2.5
	S.E.	2.07	.80	4.77	2.43	1.86	1.25	1.05	1.88	.90
	t	.79*	-4.16	-1.59*	-3.24	.05*	.59*	1.96*	2.69	2.80
Total	n	49	49	44	49	48	48	48	48	49
	$\bar{x}$	24.4	-18.7	-8.4	-9.0	2.1	-4.9	-1.7	11.3	12.2
	S.E.	4.87	2.73	1.22	1.40	.88	1.36	1.24	1.42	1.76
	t	5.01	-6.84	-6.88	-6.43	2.33	-3.61	-1.33*	7.94	6.90

* An asterisk has been placed on those values not significantly different from zero (95%) level.

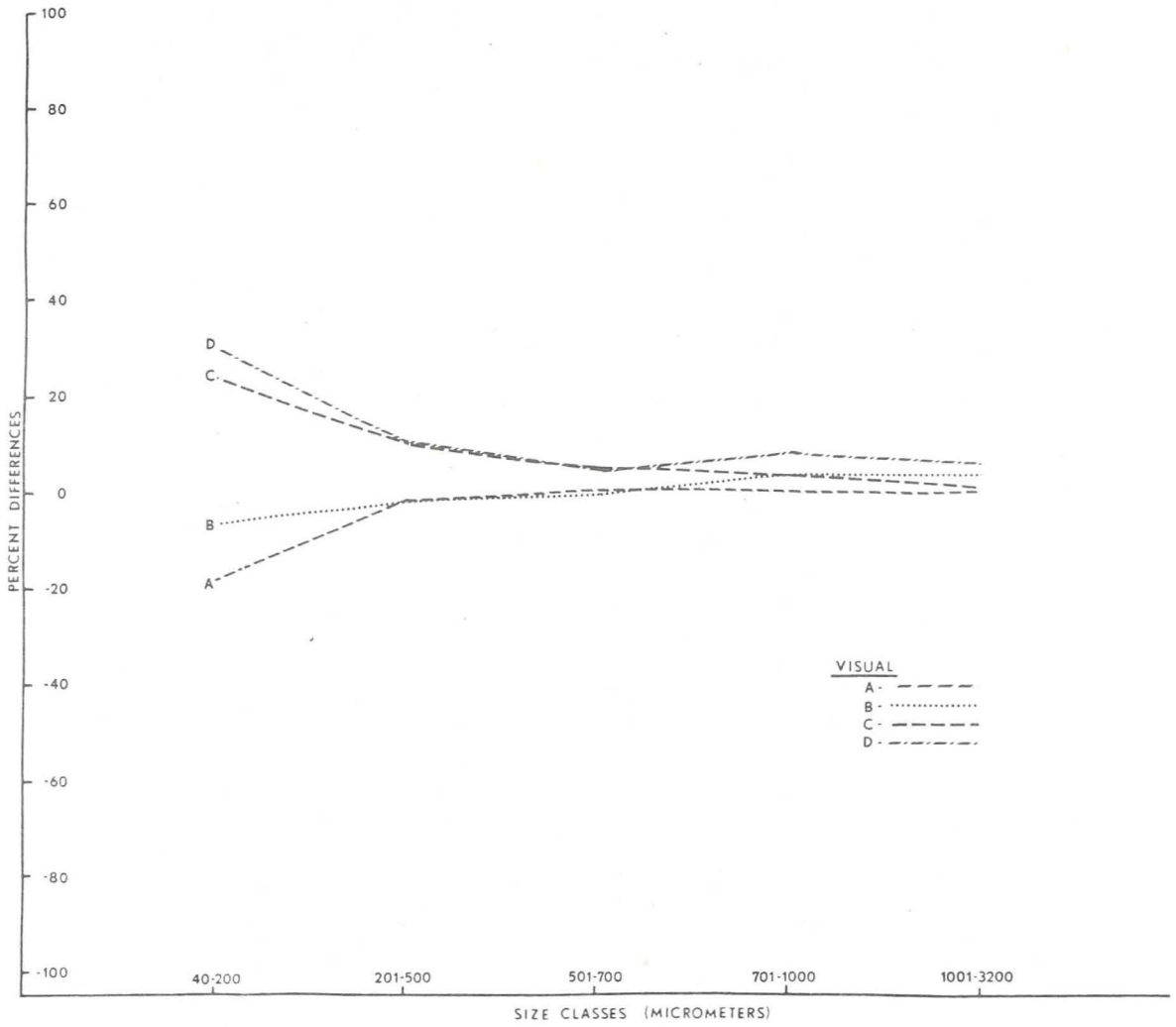

FIGURE 2. - PERCENT DIFFERENCES FROM THE DERIVED STANDARD SIZE - VISUALLY SIZED OBSERVATIONS.

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APPENDIX

Configuration of Quantimet 720 Image Analyzer for Counting and Sizing Stains on Kromekote Cards.

(1) Image System. - Macroviewer or Microscope
(2) Scanner Type. - Vidicon or Plumbicon
(3) System Control and Display. - These are an integral part of the system used for analysis of optically derived images.
(4) "Shading" Compensation. - This is an automatic matrix shading corrector. Non-uniformities which arise from less-than-perfect illumination, optics, and variations of sensitivity across the face of the scanner tube give rise to a condition known as "shading". This has the effect of representing the contrast of a given stain slightly different depending on its position in the field of view. For maximum accuracy over the whole field of view, this effect is corrected by this optional component.
(5) Feature Selector. - Known as the Detector Module, it selects the stains according to their gray level. Three different types of detectors are available. For Kromekote card assessment, a one or two dimensional detector is recommended.
(6) Variable Frame and Scale Module. - This unit enables the operator to precisely choose any region within the scanned area for measurement. An electronic "mask" or "window" permits the computing logic to analyze only those features whose "marker flags" lie within the chosen frame. The frame also eliminates counting and sizing errors. An electronic graticule may also be displayed by this unit to facilitate the calibration of the system.
(7) Choice of Sizing Logic. - The simplest method of sizing features is by measuring their maximum chord length in the horizontal (scanning) direction. For circular features this is perfectly adequate. Most Kromekote cards have stains which are elongated or oval shaped to varying degrees. Thus, the maximum horizontal chord measurement being orientation dependent, is not an accurate measure of the size of all stains. Fortunately, the Quantimet can be provided with additional computing modules which can generate data relating to the areas of individual stains. This measurement is, of course, orientation independent; therefore, a system employing Area Sizing Logic would be recommended.
(8) Elimination of Errors Caused by Clusters or "Touching" Stains. - The simplest forms of counting logic cannot always differentiate between agglomerates of features and single features. Each type would be counted as one feature, as the machine views a cluster of stains as a single feature.

However, since the shape of an agglomerate is different to that of a single feature, this can be used as a basis for differentiation. Data from clusters can then be ignored through this "Pattern Recognition" logic.

If "liters/hectare" or equivalent computations are to be derived, it may not be acceptable to ignore the contribution of the data from clusters of stains.

The best alternative approach, therefore, would be to use the operator-interactive "Image Editor." This unit is a special light-pen which permits the operator to "cut" clusters of stains into their component parts by drawing on the display screen.

(9) Data Handling. - The selection of choices described above depends largely upon the speed of operation required and the amount of data handling to be performed on-line. Criteria must be established prior to the assessment relative to the level of precision which is required.

In summary, the preferred Image Analysis System for deposit cards may incorporate the following:

(1) Macroviewer or microscope
(2) Vidicon or Plumbicon Scanner
(3) System Control and Display
(4) Automatic Matrix Shading Corrector
(5) Detector Module
(6) Variable Frame and Scale
(7) Area Sizing Logic Modules
(8) Image Editor Light-Pen
(9) Data Handling as Selected by the User